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REVIEW

Interactions of arbuscular mycorrhizal fungi, critical loads of nitrogen deposition, and shifts from native to invasive species in a southern California shrubland[1]

Edith B. Allen, Louise M. Egerton-Warburton, Bridget E. Hilbig, and Justin M. Valliere

Abstract: Anthropogenic nitrogen (N) deposition and invasive species are causing declines in global biodiversity, and both factors impact the diversity and functioning of arbuscular mycorrhizal (AM) fungi. Shifts in arbuscular mycorrhizal fungal (AMF) communities can generate feedback to native plants and affect their success, as was observed in California's coastal sage scrub, which is a Mediterranean-type shrubland threatened by invasive grasses. As vegetation-type conversion from native shrubland to exotic annual grassland increased along a gradient of increasing N deposition, the richness of native plant species and of spore morphotypes decreased. Rapid declines in all plant and fungal values occurred at the critical load (CL) of 10–11 kg N·ha^{-1}·year^{-1}, indicating that AM fungi respond to the same environmental signals as the plants, and can be used to assess CL. Shrub root colonization also decreased along the N gradient, but colonization of the invasive grass was dominated by a fine AMF endophyte that was unresponsive to elevated N. A greenhouse experiment to assess AMF functioning showed that the native shrub *Artemisia californica* Less. had a negative growth response to an inoculum from high-N but not low-N soils, whereas the invasive grass *Bromus rubens* L. had a positive response to both inocula. Differential functioning of AM fungi under N deposition may in part explain vegetation-type conversion and the decline of this native shrubland.

Key words: fine endophyte, *Glomus tenue*, nitrogen critical load, vegetation-type conversion.

Résumé : Le dépôt d'azote (N) anthropique et les espèces envahissantes sont responsables du déclin de la biodiversité globale, et les deux facteurs affectent la diversité et le fonctionnement des champignons mycorhizes arbusculaires (CMA). Des changements dans les communautés de CMA affectent aussi le succès des plantes indigènes, comme observé dans les buissons de sauge côtière de Californie, des arbustaies de type méditerranéen menacées par les herbes envahissantes. Alors que la conversion du type de végétation, d'une arbustaie indigène vers une prairie d'annuelles exotiques, augmentait en fonction d'un gradient d'augmentation de dépôt de N, la richesse en espèces de plantes indigènes et en morphotypes de spores de CMA diminuait. Les déclins rapides de toutes les plantes et unités fongiques survenaient à une charge critique (CC) de 10–11 kg N·ha^{-1}·année^{-1}, indiquant que les CMA répondent aux mêmes signaux environnementaux que les plantes et qu'ils peuvent être utilisés pour évaluer la CC. La colonisation racinaire des arbustes diminuait aussi en fonction du gradient de N, mais la colonisation des herbes envahissantes était dominée par un endophyte CMA fin qui ne répondait pas à des quantités élevées de N. Une expérience réalisée en serre visant à évaluer le fonctionnement des CMA a montré que l'arbuste indigène *Artemisia californica* Less. présentait une réponse de croissance négative à l'inoculum des sols riches en N, mais pas des sols pauvres en N, alors que l'herbe envahissante *Bromus rubens* L. présentait une réponse positive aux deux inocula. Le fonctionnement différentiel des CMA en fonction du dépôt de N peut expliquer en partie la conversion du type de végétation et le déclin de cette arbustaie indigène. [Traduit par la Rédaction]

Mots-clés : endophyte fin, *Glomus tenue*, charge critique d'azote, conversion du type de végétation.

Introduction

Anthropogenic nitrogen (N) deposition has recently been documented as a cause of decline in shrub and grassland ecosystems (Bobbink et al. 2010; Fenn et al. 2003, 2010; Rao et al. 2010; Cox et al. 2014). These ecosystems are subject to loss of native plant diversity (Stevens et al. 2010) and vegetation type conversion to exotic annual grasslands (Fenn et al. 2003, 2010; Rao et al. 2010;

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E.B. Allen, B.E. Hilbig, and J.M. Valliere. Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA 92521, USA.
L.M. Egerton-Warburton. Chicago Botanic Garden, Plant Science Center, 1000 Lake Cook Road, Glencoe, IL 60022, USA.
Corresponding author: Edith B. Allen (email: edith.allen@ucr.edu).

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Cox et al. 2014). Such conversions may be mediated by the direct effects of N fertilization on plants with traits that make some species more productive or competitive than others (Suding et al. 2005; Dirnbock et al. 2014), or indirectly through the effects of N on mycorrhizal fungi. Elevated N causes decreases in arbuscular mycorrhizal fungal (AMF) root colonization and spore production (Egerton-Warburton and Allen 2000; Johnson et al. 2003; Egerton-Warburton et al. 2007; van Diepen et al. 2011), and a predominance of poor AMF mutualists that promote reduced growth of host plants (Johnson 1993).

Invasive species increase under N deposition when they are more responsive to N than native species (Rao and Allen 2010). These invasive species have direct impacts on native species composition (Cox and Allen 2008; Steers et al. 2011), which may also be mediated by mycorrhizal fungi (Marler et al. 1999; Pringle et al. 2009). Furthermore, there is feedback between invasive species and soil microbes, and the effects include shifts in AMF species composition and diversity reduction (Mummey and Rillig 2006; Sigüenza et al. 2006*a*; Barto et al. 2011; Busby et al. 2013; Hilbig and Allen 2015). Shifts in AMF species composition have a feedback effect on the hosts that involves changing the physiological response of their host plants; futhermore, plant species' responses to AMF infection vary within a plant community, depending on the species of arbuscular mycorrhizal (AM) fungi and the dependence of the plant species on AM fungi (Allen et al. 2003; Klironomos 2003).

In southern California, which receives high levels of N deposition due to air pollution (Fenn et al. 2010), N deposition has been implicated in the widespread conversion of coastal sage scrub (CSS) to exotic annual grasslands (Allen et al. 1998; Fenn et al. 2010; Cox et al. 2014). Coastal sage scrub is a drought-deciduous Mediterranean-type shrubland with high species richness and endemism that has been reduced to a small fraction of its historic range as a result of land-use change, invasive species, and increased fire frequency. It is one of the most endangered ecosystems globally, with losses in plant species richness across much of its range (Cleland et al. 2016). The role of mycorrhizae in the interactions between exotic annual grasses and native shrubs has already been tested (Yoshida and Allen 2004; Sigüenza et al. 2006*b*), but the role of AM fungi in the shift to exotic annual grasslands has not been considered on the landscape scale, and will be considered here.

Furthermore, the decline in AM fungi, similar to the decline in native plant species richness, may be used as an indicator of critical load (CL) for N deposition. The CL for N deposition is that amount of N below which there are no negative impacts on an ecosystem (Pardo et al. 2011). These can be measured as changes in structure (e.g., species richness, vegetation-type conversion) or function (e.g., mycorrhizal functioning, increased fire due to invasive species). Cox et al. (2014) calculated a CL of 11 kg N·ha^{-1}·year^{-1} for the shift from native CSS to exotic annual grassland dominated by Mediterranean invasive grasses, but this study focused on vegetation rather than AM fungi.

During the last 20+ years, we have undertaken a series of studies to understand how anthropogenic N deposition affects plants and the AM fungi of CSS. The objectives of this review are to synthesize previous studies on the relationships between N deposition, native and invasive species, and AMF activity, especially pertaining to CSS, and to compare this information with a more recent analysis of CLs for N deposition in CSS (Cox et al. 2014). The specific objectives are to review (*i*) the impacts of N deposition on the abundance of native and invasive species of CSS in California; (*ii*) the impacts of N deposition on AMF diversity and activity; (*iii*) the impacts of the CLs for N deposition on AM fungi, and relate these to CLs for vegetation-type conversion; and (*iv*) functional responses of native and invasive species to shifts in AMF diversity and to elevated N.

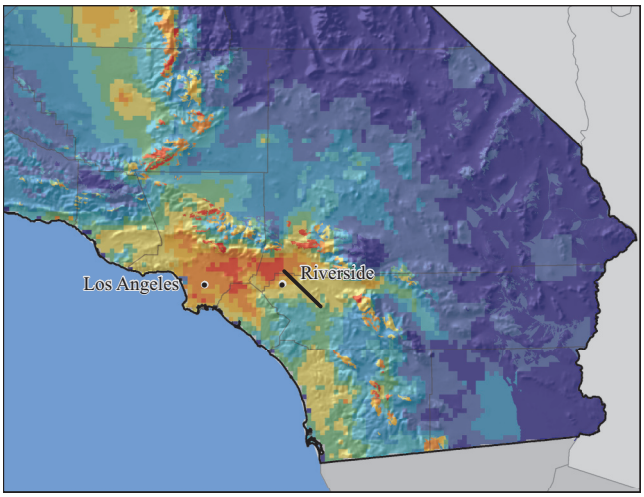
Nitrogen deposition gradient in CSS

Before proceeding with examining the impacts of N deposition on AM fungi and their associated CSS vegetation, we review the N deposition values that occur in this vegetation type. After the EPA Community Multiscale Air Quality (CMAQ) model of N deposition for California became available (Fenn et al. 2010), we were able to calculate the CLs of N deposition for impacts on plants and AM fungi. The California version of the CMAQ was generated at a 4 km × 4 km scale for 2002, which provides finer resolution than the 12 km × 12 km national CMAQ model. The N deposition values in the southern California CSS range from 2 to 20 kg N·ha^{-1}·year^{-1} (Allen et al. 2014), with 2 being the background level in unpolluted areas, and 20 the highest value in wildland areas downwind of urban Los Angeles. The range of high values across the US is also >20 kg N·ha^{-1}·year^{-1} (NADP 2013), so values for N deposition in CSS are as high as any found in the US. By contrast, the highest values in a European grassland N deposition gradient were near 40 kg N·ha^{-1}·year^{-1} (Stevens et al. 2010), i.e., higher than the recorded US values. Both oxidized and reduced forms of N are deposited, as nitrate/nitric acid from internal combustion, and ammonia/ammonium from agriculture and catalytic converters (Fenn et al. 2010). About 90% is deposited in the dry form during the long summer dry season in this Mediterranean-type climate, and accumulates on soil surfaces (Padgett et al. 1999; Bytnerowicz et al. 2016). The N deposition gradient we studied was in CSS in western Riverside County, and ranged from 8.7 to 19.6 kg N·ha^{-1}·year^{-1} (Fig. 1).

Growth of individual CSS plants under elevated N

Elevated N preferentially increases the growth and rate of N uptake of the dominant invasive grass *Bromus rubens* L. (= *B. madritensis* subsp. *rubens*) over the dominant native shrub it is displacing, *Artemisia californica* L.

Fig. 1. Nitrogen deposition in western Riverside County, southern California, showing a gradient 60 km long that ranges from 8.7 to 19.6 kg N·ha^{-1}·year^{-1} and lies within coastal sage scrub vegetation. Map created by Robert Johnson, Center for Conservation Biology, University of California at Riverside.



(Yoshida and Allen 2001, 2004). When individuals of *B. rubens* and *A. californica* were grown individually in pots in sterile soil, growth rates of both species were similar, although the mortality of *A. californica* was high at the highest level of N fertilization (Padgett et al. 1999). However, when individuals were grown in nonsterile soil, AMF-colonized roots of *B. rubens* acquired labeled $^{15}NH_4^+$ (but not $^{15}NO_3^-$) at a higher rate than *A. californica* (Yoshida and Allen 2004). This suggests that *B. rubens* can take up N from NH_4^+-dominated field soils (most of the soils in the region are slightly acidic in pH and NH_4^+-dominated; Padgett et al. 1999). However, these growth results for individual plants in pots are insufficient to explain the role of N deposition and AM fungi in the shift from shrubland to grassland, and further field and greenhouse studies are presented that are more revealing.

Plant community shifts in response to N fertilization and implications for AM fungi

Studies in Californian grasslands have shown that N fertilization or N deposition increases the abundance of exotic annual grasses preferentially over native grasses and forbs (Huenneke et al. 1990; Weiss 1999). Similarly, invasive grasses increased in abundance in southern Californian CSS with increased N availability (Figs. 2*a* and 2*b*). CSS vegetation was fertilized, beginning in 1994, with 60 kg N·ha^{-1}·year^{-1} as NH_4NO_3, following a wildfire in 1993 in an area of relatively low N deposition (Figs. 2*a* and 2*b*). The biomass of exotic annual grasses (predominantly *B. rubens*) increased in N-fertilized plots, whereas the cover of native shrubs, dominated by *A. californica*, decreased. As this community reassembled from bare ground following the fire, competition from productive, N-fertilized *B. rubens* likely reduced shrub seedling establishment, as was previously demonstrated in a grass removal experiment (Eliason and Allen 1997). However, AM fungi may also be implicated, as AMF colonization of native shrub roots was lower in plots with elevated levels of N (Egerton-Warburton and Allen 2000; Sigüenza et al. 2006*b*), but there was no significant difference in AMF colonization of roots between the N-fertilized and unfertilized *B. rubens* in these plots (Sigüenza et al. 2006*b*). Loss of AM fungi may negatively impact the hyphal network that promotes water transport to shrubs during the summer dry season (Allen et al. 2010; Querejeta et al. 2012), further reducing their growth.

Critical load for loss of plant richness on an N deposition gradient

The N fertilization experiments reported above confirmed the patterns in native and invasive species shifts on the N deposition gradient, where native species richness declined from 67 to 15 species at between 8.7 to 19.6 kg N·ha^{-1}·year^{-1} (Allen et al. 2014; Table 1). Based on the steep drop in species richness between 9.6 and 11 kg N·ha^{-1}·year^{-1}, we estimated that the CL for species richness loss is ~10 kg N·ha^{-1}·year^{-1}. Percent cover of native forbs and shrubs also declined steeply along this gradient, whereas the cover of invasive annual grasses increased (Table 1). The low level of N deposition we observed in this CSS gradient is not the lowest level of N found in southern California, and precludes testing the response at lower levels at this site. For instance, the impacts of N deposition on plant species richness in European grasslands were reported by Stevens et al. (2010), who observed species losses over an N deposition range of 2 to 40 kg N·ha^{-1}·year^{-1}. However, lower levels of N deposition occur in the Venturan (coastal) association of CSS, and this N gradient is the topic of current research in the Santa Monica Mountains. Studying a gradient with lower N deposition may result in a lower CL for loss of species richness.

This observation of CLs for loss of plant species richness is based on relatively few sites in which a steep drop in richness occurs above 9.6 kg N·ha^{-1}·year^{-1}. To confirm this value, a more rigorous approach to estimate CL was taken by quantifying vegetation-type conversion from native shrubland to exotic annual grassland (Cox et al. 2014). A vegetation survey from 1930 was compared with a modern resurvey from 2002. The analysis of 150 sites showed that 35% of CSS converted to exotic grassland within 72 years, and mostly in areas with high N deposition. The probability of type-conversion to exotic grassland increased steeply at 11 kg N·ha^{-1}·year^{-1} (Cox et al. 2014). We took this to be the CL for vegetation-type conversion, similar to the CL for richness loss (Table 1).

AMF activity across N gradients as indicators of critical load

A similar loss of AMF spore morphospecies, as well as reduced percent root colonization and spore density in rhizospheres of native CSS shrubs (*A. californica*, *Encelia farinosa*, and *Eriogonum fasciculatum*) occurred on this N

Fig. 2. (*a*) Percent cover of native shrubs following a fire in 1993, in coastal sage scrub, in plots that were fertilized with nitrogen and the control plots. (*b*) Biomass of the exotic grass *Bromus rubens* in the control and N-fertilized treatments. *P*, overall significance for repeated measures analysis; *, $P < 0.05$ for individual dates (E. Allen, unpublished data).

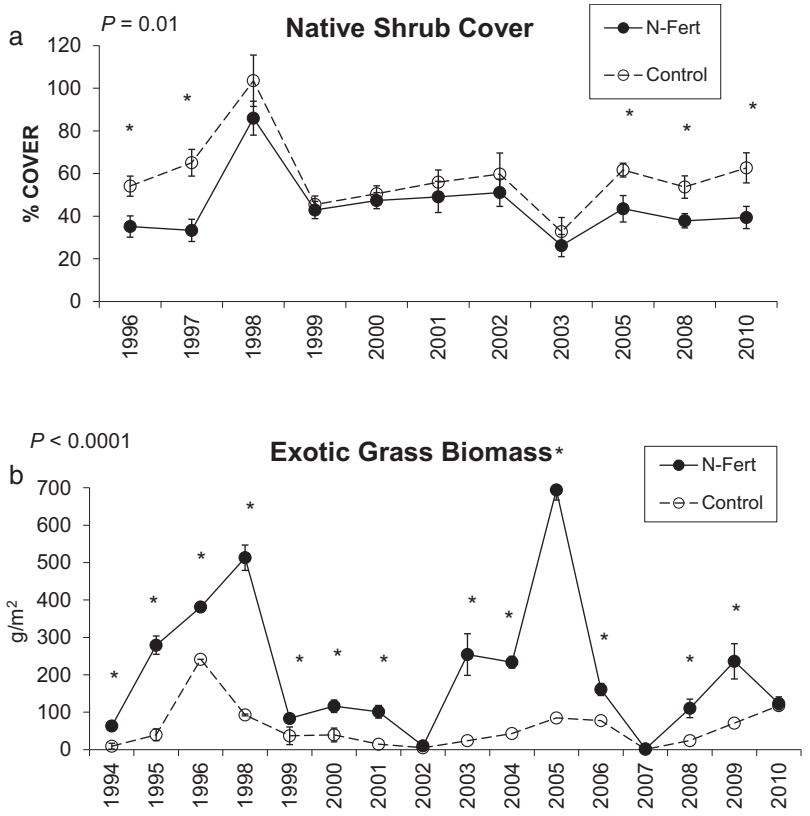


Table 1. Percent cover and richness (number of species per 3 ha) of plant groups along a nitrogen (N) deposition gradient in western Riverside County.

Site	Exotic grass (% cover)	Native forb (% cover)	Shrub (% cover)	No. of native forb species	Soil N ($\mu g \cdot g^{-1}$)	N deposition ($kg\ N \cdot ha^{-1} \cdot year^{-1}$)
Jurupa Hills	63.5	4.0	2.2	16	37.7	19.6
Box Springs	69.2	18.5	2.4	31	32.6	14.7
Botanic Garden	36.0	25.4	0.2	20	28.9	13.4
Lake Perris	0.5	26.1	2.8	30	20.3	11.1
Mott Reserve	6.7	14.3	11.2	**37**	30.6	**11.1**
Lopez Canyon	11.1	19.6	19.3	**67**	9.6	**9.0**
Tucalota Hills	1.5	35.7	35.0	50	10.5	8.7

Note: Sites are arranged from north to south along an urban to rural gradient. Forb species richness in bold shows a rapid drop in richness, suggesting a critical load of 10 $kg\ N \cdot ha^{-1} \cdot y^{-1}$ based on the CMAQ model of N deposition (Fenn et al. 2010).

gradient (Egerton-Warburton and Allen 2000; Tables 2 and 3). Root colonization declined from 45% to 19%, and spore density declined from 105 to 23 spores·(g soil)$^{-1}$ with elevated N (Table 2). A total of 24 species of AM fungi were observed, with 19 being the highest number at any site (Table 3; Egerton-Warburton and Allen 2000). Spore species richness declined from 19 to 11 per site across the N gradient. The greatest decline was in the 8 species of large-spored genera (*Scutellospora* [3 species] and *Acaulospora* [2 species], as well as *Gigaspora*, *Entrophospora*, and *Sclerocystis* [1 species each]), which declined from a total of 37% to 7% of the total species richness with elevated N (Table 3). The remaining 16 species were all *Glomus* spp. *Glomus aggregatum* was the most abundant species (by density) at all sites, and increased further with elevated N. Other *Glomus* species either declined in density with elevated N or showed no discernible pattern in response to N (Egerton-Warburton and Allen 2000).

The declines in AMF species richness and percent colonization were primarily a function of elevated N rather than host species (shrub vs. grass). We believe this to be the case because soil and root samples were collected under shrubs that occurred in remnant patches, even in regions with high N deposition. Rhizosphere soils of grasses were not collected for this study (Egerton-Warburton and Allen 2000). Furthermore, exotic grasses occur throughout

Table 2. Spore counts and percent root colonization of arbuscular mycorrhizal fungi along a nitrogen (N) deposition gradient in western Riverside County.

Site	Spores·(g soil)$^{-1}$	Root colonization (%)	Soil N ($\mu g \cdot g^{-1}$)	N deposition (kg N·ha^{-1}·year^{-1})
Jurupa Hills	23	19	86.2	19.6
Waterman Road	54	18	15.6	16.9
Box Springs Mountain	66	17	26.4	14.7
Mockingbird Canyon	74	24	56.8	12.5
Lake Mathews	70	23	39.5	**11.1**
Motte Reserve	80	27	26.1	**11.1**
Hemet	**78**	**22**	12.8	**9.6**
Lake Skinner	**103**	**39**	7.5	**8.7**
Santa Margarita	105	45	9.7	8.7

Note: Sites are arranged from north to south along an urban to rural gradient (data from Egerton-Warburton and Allen 2000). Values in bold show a drop in spore density and root infection, and are interpreted to represent a critical load.

Table 3. Diversity of arbuscular mycorrhizal fungal (AMF) spores decreased with increasing extractable soil nitrogen (N) along the nitrogen deposition gradient of western Riverside County.

Site	Santa Margarita	Lake Skinner	Hemet	Motte Reserve	Lake Mathews	Mockingbird Canyon	Box Springs Mountain	Jurupa Hills
Soil N ($\mu g \cdot g^{-1}$)	9.7	7.5	12.8	26.1	35.0	39.5	26.4	86.2
N deposition (kg N·ha^{-1}·y^{-1})	8.7	8.7	9.6	11.1	12.5	12.5	14.7	19.6
Diversity (H')	0.766	0.564	0.269	0.422	0.579	0.315	0.163	0.243
AMF species (n)	19	19	11	18	15	14	14	12
Large-spored taxa (n)	7	4	2	4	4	2	1	1
Large-spored taxa (%)	36.8	21.1	18.2	22.2	26.7	14.3	7.1	8.3

Note: Many large-spored species failed to reproduce in high-nitrogen soil (Egerton-Warburton and Allen 2000). Modeled nitrogen deposition values are from Fenn et al. (2010).

the N gradient, although more extensively across the landscape under elevated N deposition. A reduction in shrub root colonization with elevated N may be triggered by molecular signaling and reduced provisioning of photosynthate to AM fungi (reviewed in Egerton-Warburton and Allen 2000; Johnson et al. 2003). Soils of the Californian CSS are relatively high in phosphorus (30 $\mu g \cdot$(g soil)$^{-1}$; Padgett et al. 1999). Reduced mycorrhizal activity following N eutrophication is especially pronounced in soils with initially low N:P ratios, such that elevated N causes both N and P to be in high supply (Johnson et al. 2003). Preferential loss of large-spored species with elevated N may be related to their greater carbohydrate demand for reproduction (reviewed in Egerton-Warburton and Allen 2000; Egerton-Warburton et al. 2007).

The patterns in reduction of AMF activity are largely similar to those for loss of plant species richness under N deposition. The CL for decline of AMF activity is above 9.6 kg N·ha^{-1}·year^{-1} (Tables 2 and 3). This corresponds to the decline in plant species richness above 9.6 kg N·ha^{-1}·year^{-1} (Table 1) and vegetation type conversion at ~11 kg N·ha^{-1}·year^{-1} from native shrubland to exotic annual grassland (Cox et al. 2014). Both the decreased shrub AMF activity and loss of plant species richness are related to increased soil N, but native plant richness decline is also related to increased invasive grass productivity. This suggests that AMF spore richness and percent infection of shrub roots can be used as indicators of elevated N, as they declined where there were few remaining shrubs, such as found in the high deposition sites (Egerton-Warburton and Allen 2000). Alternatively, grasses tend to form associations with fine AM fungi (Siguenza et al. 2006*a*, 2006*b*; Hilbig and Allen 2015), which might cause a decline in spore richness even in the absence of N deposition. However, we did not quantify spore richness for grass rhizospheres. In support of our shrub rhizosphere analysis, a retrospective study using archived soil samples from a higher elevation chaparral site in the Los Angeles air basin showed a decline in density of all spore species and a loss of large-spored genera between 1937 and 1961, with further declines through 1999 (Egerton-Warburton et al. 2001). In this site, soil $\delta^{15}N$ values (an indicator of N emissions from internal combustion) had been elevated since at least 1958. As a result, N deposition has affected AMF activity long enough to cause feedback to the plant community.

Feedback between N-fertilized AMF inoculum and invasive and native plant species

Another analysis of AMF activity was done along the same N deposition gradient (Egerton-Warburton and Allen 2000), which included the invasive grass *B. rubens* as well as the native shrub *A. californica* (Sigüenza et al. 2006*b*). Spore counts (richness was not assessed) in the

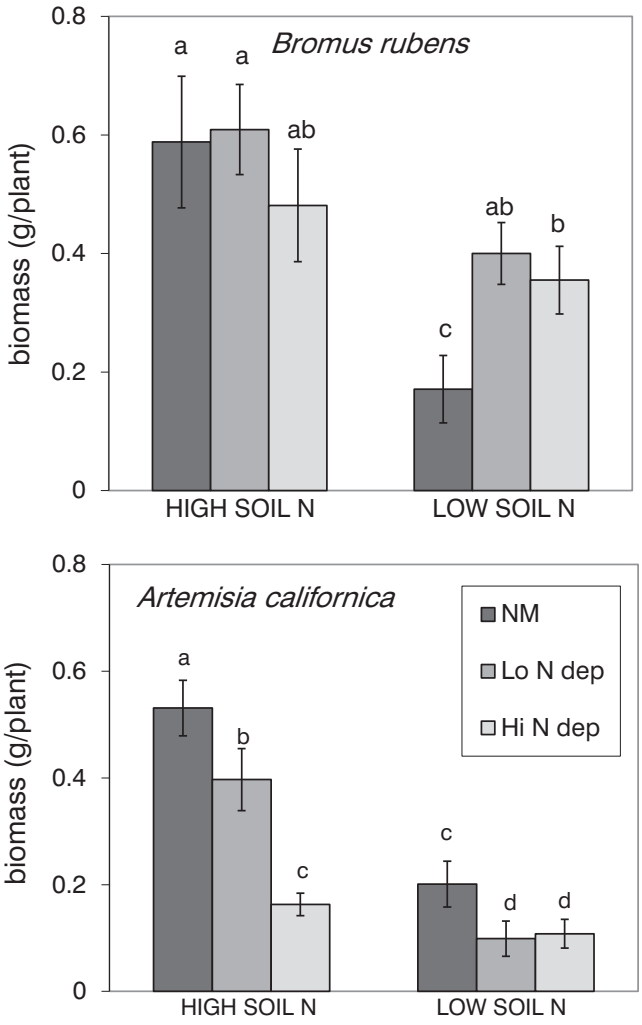
rhizosphere of the invasive grass did not decline significantly with elevated soil N, whereas spore counts of the native shrub decreased; the latter was also observed by Egerton-Warburton and Allen (2000). Furthermore, approximately 50% of *B. rubens* root colonization was by a fine AMF endophyte, *Glomus tenue* (with up to 80% total root infection, sum of coarse plus fine endophyte). In contrast, *A. californica* roots showed only traces of fine AMF colonization (Sigüenza et al. 2006*b*), and *G. tenue* spores and root infection were not observed by Egerton-Warburton and Allen (2000) in the shrub rhizospheres. The next step was to determine whether there is a functional response of mycorrhizal fungi involved in the success of *B. rubens* and the poor performance of *A. californica* under N deposition.

We report on a greenhouse study used to demonstrate the effect of feedback of N-deposition-altered AMF communities on the growth of *B. rubens* and *A. californica* (Sigüenza et al. 2006*a*). Three inoculum treatments from this study were (A) soil inoculum from a low N deposition site (8 kg N·ha^{-1}·year^{-1}); (B) soil inoculum from a high N deposition site (20 kg N·ha^{-1}·year^{-1}); and (C) sterile soil (sterilized inocula from high deposition and low deposition sites to balance the nutrients). Each of the inoculum treatments was given a high or low N fertilizer treatment. The potting mix was soil from the low N deposition site, and the N fertilization treatment was supplemented with NH_4NO_3 to increase N to the levels found in soils of the high N deposition site.

Bromus rubens responded positively to both inoculum sources by comparison with the sterile controls in low-N soil, but there were no significant effects from the inoculum in high-N soil (Fig. 3*a*). Conversely, *A. californica* seedlings had a negative response to the inocula from both high and low N soils, but the inoculum from the high N deposition soil was especially detrimental to its growth. The greenhouse study of Sigüenza et al. (2006*a*) was done under a moderate water regime, i.e., pots were watered to field capacity and surface soils allowed to dry without causing the seedlings to wilt. Recently, we performed a variation on this study by watering pots to field capacity or limiting soil moisture to simulate drought stress in *A. californica* seedlings, and found that inoculum from high N deposition soil was especially detrimental under drought stress (Valliere and Allen 2016).

In a greenhouse study by Sigüenza et al. (2006*a*), as in the field study (Sigüenza et al. 2006*b*), *B. rubens* was colonized by fine AMF hyphae, likely *G. tenue*, whereas *A. californica* was colonized by coarse hyphae. The negative response of *A. californica* seedlings to AMF colonization may be related to the high P levels (30 μg·(g soil)$^{-1}$; Padgett et al. 1999) of these soils, as high P levels may cause growth reduction in mycorrhizal plants, especially at the seedling stage (e.g., Johnson et al. 1997; Allen et al. 2003). Alternatively, *G. tenue* may have promoted a positive growth response in *B. rubens* in these high-P soils

Fig. 3. Greenhouse growth responses of the invasive grass *Bromus rubens* and the native shrub *Artemisia californica* to soil inocula from sites with low (Lo N dep) or high (Hi N dep) nitrogen deposition compared with sterile soil (NM, nonmycorrhizal) under two levels of levels of soil fertilization with nitrogen. Different letters indicate statistically significant differences ($P < 0.05$). Image redrawn using data from Sigüenza et al. (2006*a*).



under conditions of low N, relative to the sterile controls. When levels of N were elevated, there were no significant differences in biomass response to inoculation in the greenhouse experiments, even though there was a reduction in percent mycorrhizal colonization in the high N deposition soil (Sigüenza et al. 2006*a*). Nonmycorrhizal, potentially pathogenic fungi were also assessed microscopically in this study, and their colonization was greater for both species in one treatment only: high N deposition inoculum under high N fertilizer (Sigüenza et al. 2006*a*); so the incidence of pathogens does not explain the growth responses observed in this study. These findings suggest that *G. tenue* is an effective mutualist, even at low levels of root colonization and under high soil N availability, (Hilbig and Allen 2015). *Glomus tenue* (syn. *Rhizophagus tenuis*) is commonly found in soils of

harsh and disturbed environments (Olsson et al. 2004), and is considered to be more frequent in pioneer plants (Blaschke 1991). A positive feedback from *G. tenue* was also found to contribute to the overall success of the exotic annual *Bromus diandrus* by compensating for the negative impacts of putative pathogens in an abandoned agricultural field (Hilbig and Allen 2015).

Conclusions

By synthesizing a series of studies on AMF and plant responses with recently available data on N deposition (Fenn et al. 2010), we have shown that AM fungi may be important indicators of CLs for N deposition, and may also have an central role in the vegetation-type conversion of CSS to exotic annual grassland. Threshold losses in AMF morphospecies richness, native plant species richness, and vegetation-type conversion from native shrubland to exotic annual grassland all occurred at 10–11 kg N·ha^{-1}·year^{-1}. These losses in AMF diversity co-occurred with a decrease in AMF root colonization in *A. californica* but not *B. rubens* in field-collected samples (Sigüenza et al. 2006*a*). Furthermore, AM fungi of *A. californica* are sensitive to elevated N, producing small seedlings that are unlikely to compete with fast-growing *B. rubens* with its fine AM fungi that are insensitive to elevated N (Sigüenza et al. 2006*b*). By combining these studies, we suggest that processes occurring on very small scales of individual plant–mycorrhizal interactions can have ramifications on the landscape scale for vegetation-type conversion under the impact of N deposition.

Further evidence for AMF-induced vegetation changes may come by studying additional invasive and native species of CSS, and by expanding these studies to other ecosystem types. For instance, a closely related invasive grass, *B. tectorum*, is causing vegetation type conversion throughout *Artemisia tridentata* shrublands of the western US, even in the absence of N deposition, but most of the invasion appears to be in soils that are naturally high in N and P (Belnap et al. 2016). Losses of richness in European grasslands and shrublands is not related to invasive species, but rather to increases in native grass species under N deposition (Bobbink et al. 2010; Stevens et al. 2010). The role of AM fungi under conditions of changing nutrients has been little explored in these situations of species loss, and deserves further study.

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